Antimicrobial activity of Boscia Senegalensis plant extracts against some bacterial strains in water treatment

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Abstract

The study aimed to explore the possibility of utilizing the efficiency of these natural extracts in purifying and sterilizing drinking water, to improve water quality instead of other chemicals, by evaluating the in vitro activity of the plant Boscia Senegalensis (Pers.) and comparing the differences between the use of leaf and bark extracts against some pathogenic bacteria strains found in the drinking water of East and Center of CHAD. To achieve this, ethanolic extracts were used against three bacterial strains including Gram-negative such as Salmonella typhi (SL), Escherichia coli (EC), and Klebsiella pneumoniea (KL). The extracts showed activity with an inhibition zone for leaf and bark extracts of about (18, 24, 21), (26, 21, and 23) mm against SL, EC, and KL, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were (6.25 and 12.5) mg/ml, respectively. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone, and results were compared with a standard antibiotic (Ciprofloxacin). All leaf and bark extracts showed antibacterial and anti-fungal activity. However, bark extracts showed more advantages, possibly due to the presence of secondary metabolites in the plant.

Key words-*Antibacterial activity, Boscia Senegalensis, bark and leave Extracts, Bacteria.*

I. Introduction

Antibiotic resistance has become one of the biggest challenges for physicians and other infection control agents as they seek to prevent and treat infections caused by microorganisms once thought to be eradicated by antibiotics. These organisms, or bacteria, are returning in new forms resistant to almost all clinically important antimicrobial[1]. Salmonella bacteria" are important species of the Enterobacteriaceae family, which are chemically and serologically diverse and cause several diseases such as typhoid fever and gastroenteritis is considered fatal illness prevalent in Africa and South Asia[2]. Their main location is the intestines of animals and humans, the intestines of birds, reptiles, and sometimes some insects. The inappropriate use of antibiotics leads to the spread of antimicrobial resistance in humans. It has led to the emergence of multi-resistant Salmonella enterica strains[3][4]. Klebsiella pneumonia" is a Gram-negative responsible for a great variety of infections[5] and an intestinal bacterium that is an opportunistic pathogen that causes a wide range of diseases and exhibits frequent acquisition of antibiotic resistance Escherichia coli" bacteria colonize the gastrointestinal tract of human infants shortly after birth. E. coli bacteria have coexisted with humans in good health and have had mutual benefits for decades.[6] Many highly adaptive E. coli clones have acquired characteristics that give them an increased ability to adapt to new environments and allow them to cause a wide range of diseases. These bacteria can now cause illnesses in healthy people. The following disease syndromes can arise: enterocolitis/diarrhea, sepsis/meningitis, and urinary tract infections[7]. There is a great need for healthy water with the increase in population. Surface water, such as lakes, and reservoirs, cannot be consumed because of its pollutants by organic materials, and pathogenic microorganisms. Most rural areas in Chad suffer from the lack of drinking water treatment. It was found practically, after several experiments, that much surface water suffers from biological contamination with bacteria (Salmonella typhi, total coliforms). Usually, biological treatment is achieved using chemical disinfectants such as chlorine and ozone or radiation such as UV, which are not available in those areas due to their cost, Already, areas used some plants as a natural coagulant to reduce the turbidity from drinking, the common plants were used (Moringa oleifera, Okra, Acorn, Aloe Vera, Boscia Senegalensis) In a previous study, used Boscia S. (Pers.)Lam. ex Poir plant as Coagulants proved the efficiency in reducing the turbidity it was obtained Efficiency to reduce the turbidity from drinking water. This study aimed to study Boscia S. to evaluate the in vitro activity against some pathogenic bacteria. Several authors report antibacterial activity, Boscia S. plant is not toxic, traditionally used for animal diseases, and has higher antioxidant activities.[8]

II. Materials and Methods

A. Materials

The materials used were placed in Table(I) showing their specification and Manufacturer, three Media used were Mueller Hinton agar for the sensitivity test, Mueller Hinton broth for the determination of Minimum Inhibitory Concentration (MIC), and Nutrient agar for the determination of Minimum Bactericidal Concentration (MBC). also in extraction

were used ethanol and the plant Boscia S. prates (Leaves, Barks). The test organisms used for this analysis were clinical isolates of bacteria from the Department of Microbiology, Ahmadu Bello University, Zaria, NAIJERIA. Were: Salmonella typhi- Escherichia coli- Klebsiella pneumoniea.

B. Methods

1) Extraction Method (Cold Maceration):: The pulverized samples (100 g) were macerated with 1 L of ethanol, allowed to stand for 72 hours, and filtered. The filtrate was concentrated using a rotary evaporator and evaporated to dryness in a water bath at 50°C. The dried extracts were properly stored in a container before experiments and analysis.[9].

2) Culture media: The culture media used include Mueller Hinton agar (MHA) in figure(1), Nutrient agar (NA), and Mueller Hinton broth (MHB). The media were used for sensitivity tests and determination of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration). The media were prepared according to the manufacturer's instructions and sterilized by autoclave at 121° C for 15 minutes.

3) Determination of inhibitory activity (sensitivity test) of the extract using agar well diffusion method: The standardized Inocula of both the bacterial and fungal isolates were streaked on sterilized Mueller Hinton agar plates. 4 wells were punched on each inoculated plate with a sterile cork borer. The wells were labeled according to different concentrations of the plant extracts, which were (100, 50, 25, and 12.5) mg/ml, each well was filled up with 0.2 ml of the plant extracts. The inoculated plates were allowed to stay on the bench for 60 minutes; to enable the extract to diffuse on the agar. The plates were then incubated at 370°C for one day At the end,the plates were observed for inhibition which will appear as a zone devoid of growth around the wells (inhibition zone). Ciprofloxacin was used as control the zone was measured using a ruler calibrated in millimeters to record the result

Determination of minimum (inhibitory /bactericidal) concentration MIC/MBC: The minimum inhibitory concentration of the extract was determined using tube dilution methods using Mueller Hinton broth (MH) as the diluent. The lowest concentration of extract that showed inhibition of the bacteria when the extract was tested during susceptibility testing was serially diluted in test tubes. Standard organisms were added to each tube containing the broth and plant extract. The inoculated tubes were then incubated at 370°C for 24 h. At the end of the incubation, the tubes were examined for the presence or absence of growth using a turbidity test, the lowest concentration in the series without visible sign of growth (turbidity) was considered the minimum inhibitory concentration (MIC). The MIC was used to determine the minimum bactericidal concentration (MBC) of the extract. A sterile loop was dipped into the test tubes that did not show turbidity (clear) in the MIC test and a complete loop was taken and placed on sterile nutrient agar plates. The plates were incubated at 370°C for (18–24) h. At the end of the incubation, the plates were examined for the presence or absence of growth. This was to determine whether the antimicrobial effect of the extract was antibacterial or bactericidal[10].



Fig. 1. The culture media used include Mueller Hinton Agar

C. The plant Collection

The samples of the Boscia S. plant were collected from a rural area northeast of Abéché, CHAD. the scientific names of the plants were provided by the Department of Plant Classification, University of Khartoum.

D. The equipment used in experiments

Table(II) shows the Equipment used, an Autoclave for the Sterilization of the Media, an Incubator for Incubation, and a Hot air oven for the Sterilization of the test tubes.

III. Results and Discussions

A. Results

The study used the ethanol extracts of the Boscia S. plant, (Leave and Bark) extract to evaluate the in vitro activity against bacteria of Salmonella typhi, Escherichia coli, and Klebsiella pneumoniea. This plant was selected based on its widespread use and availability,

which has not been adequately studied. The leaves of Boscia S. produced a high yield of (24, 21, and 18) mm Table(III) against bacteria of EC, KL, and SL respectively. The bark extract Table(IV) showed results of 26, 23, and 21 mm against SL, KL, and EC bacteria, respectively.figured in fig(2) The best extract was the bark extract, which achieved 26 mm against Salmonella bacteria, which is considered the closest value to the control value, which was 35 mm. Anti-bacterial activity" Tables(V andVI) show the results of determining the Minimum Inhibitory Concentration of Leaves and bark extracts (MIC). At concentrations of (12.5, 12.5, and 25), (6.25, 12.5, and 12.5) mg/ml, against SL, EC, and KL respectively, the ethanolic extract of the leaves inhibited the growth of bacteria. The results of determining Minimum Bactericidal (MBC) were observed at concentrations of leave and bark extracts (12.5, 25, and 50), (12.5, 25, and 25) mg/ml. All leaves and bark extracts had antibacterial and antifungal activity. Extracts are considered bacteriostatic, not bactericidal. However, bark extracts showed more advantages than leaves. This activity may be due to the presence of secondary metabolites in the plant such as Terpenoids, alkaloids, proteins, Saponins, carbohydrates and glycosides, flavonoids, gums and mucilage, phenolic compounds, and tannins[11].

TABLE I
THE MATERIALS USED IN EXPERIMENTS

N	The Materials	Specification	Manufacturer
1	Ethanol	99.5 %	Iganmu (Nigeria)
2	Mueller Hinton agar (MHA)	38 g/Litre (used for sensitivity test)	Himedia (India)
3	Nutrient agar (NA)	28 g/L (used for determination of MBC)	Himedia (India)
4	Mueller Hinton broth (MHB)	20 g/L (for determination of MIC)	Himedia (India)
5	Leaves of Boscia S. plant	100 g/L	Collected from (Chad)
6	Barks of Boscia S. plant	100 g/L	Collected from (Chad)
7	Salmonella typhi	a clinical isolates of bacteria	ABU, Zaria (Nigeria)
8	Escherichia coli	a clinical isolates of bacteria	ABU, Zaria (Nigeria)
9	Klebsiella pneumonie	a clinical isolates of bacteria	ABU, Zaria (Nigeria)

TABLE II
THE EQUIPMENT'S

N	The equipment's	Specification	Manufacturer
1	AUTOCLAVE	Model no; ASB 300, Serial no ; SP 21043	Astell (England)
2	INCUBATOR	Model no ; SHP100, Serial no; 20210211300012	Wincom (England)
3	HOT AIR OVEN	Model no; DSC, Serial no; 87J039	Gallenkamp (England)

B. Discussions

The main cause of diarrhea in humans and animals is bacteria, and diarrhea is the second leading cause of death among children under five in low-income countries. Antibiotics are the mainstay of treatment for diarrhea. Unfortunately, there is an increase in the number of drug-resistant bacteria[12], which has led to treatment failure and increased mortality worldwide. Because of this, some medicinal plants used in traditional medicine that have efficacy against bacterial pathogens were searched for. Two previous studies used the same plant with differences in the plant parts, and the root extract was used. (Aliou et al)[13]. and (Timitop)[14] evaluated the antibacterial activities of Boscia S. roots extracted with ethanol, water, hexane, and ethyl acetate against methicillin-resistant Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae, and Escherichia coli using agar, disk and broth diffusion assays. The extract showed activity with inhibition zone of about 19 mm, and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of (5.0 and 6.0) mg/ml, respectively.[13][15] The other extract showed activity with an inhibition from (1.0 to 13.0) mm[14][15]. Ethnopharmacological research through the previous study of (Alfred M.)[15] has shown that different extracts of species and isolated compounds from Boscia S. exhibited antibacterial, anti-fungal, and soluble epoxied hydrolyses inhibitory activities, which is in agreement with the experimental results of this study.

IV. CONCLUSIONS

After used Boscia S. plant as natural Coagulant, proved high efficiency in getting rid of the turbidity of drinking water. This study focused on the possibility of using the plant as an antibacterial against the pathogenic bacteria that are present in water, to be as double activity. Unusually this time, different parts of the plant, such as barks and leaves, have been compared. This plant can also be a good antibacterial for other bacteria other than the types of bacteria's that have been used in this study.

TABLE III
ZONE OF INHIBITION (MM) OF PATHOGENIC BACTERIA BY BOSCIA S. LEAVE EXTRACT.

Test Organisms	The concentration of extract (%)				Control Ciprofloxacin
	100	50	25	12.5	
Salmonella typhi	18	16	14	12	35
Escherichia coli	24	21	19	16	33
Klebsiella pne	21	18	15	13	37

TABLE IV
ZONE OF INHIBITION (MM) OF PATHOGENIC BACTERIA BY BOSCIA S. BARK EXTRACT.

Test Organisms	The concentration of extract (%)				Control Ciprofloxacin
	100	50	25	12.5	
Salmonella typhi	26	23	19	17	35
Escherichia coli	21	17	14	12	33
Klebsiella pne	23	19	16	14	37

TABLE V
DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (M.I.C.) AND MINIMUM BACTERICIDAL CONCENTRATION (M.B.C.)/FUNGICIDAL(M.F.C.) OF LEAVE EXTRACT AGAINST THE TEST ORGANISMS.

Concentrations of the Extract (mg/ml) for		
Test Organisms	(M.I.C)	(M.B.C)
Salmonella typhi	12.5	25
Escherichia coli	12.5	25
Klebsiella pneumoniea	25	50

No M.B.C.(Extract is bacteriostatic not bactericidal).

REFERENCES

[1] C. Y. Effah, T. Sun, S. Liu, and Y. Wu, "Klebsiella pneumoniae: an increasing threat to public health," *Annals of clinical microbiology and antimicrobials*, vol. 19, pp. 1–9, 2020.

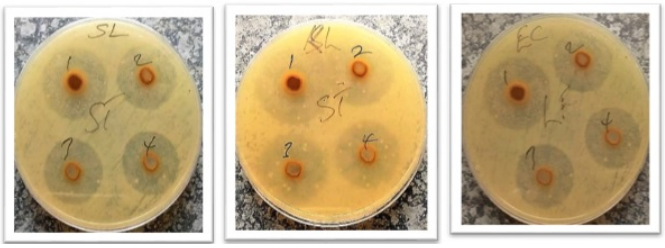


Fig. 2. Zone of inhibition of bacterial strains(SL, KL, and EC) caused by plant extracts.

TABLE VI
DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (M.I.C.) AND MINIMUM BACTERICIDAL CONCENTRATION (M.B.C.)/ FUNGICIDAL (M.F.C.) OF BARK EXTRACT AGAINST THE TEST ORGANISMS.

Concentrations of the Extract (mg/ml) for		
Test Organisms	(M.I.C)	(M.B.C)
Salmonella typhi	6.25	12.5
Escherichia coli	12.5	25
Klebsiella pneumoniea	12.5	25

[2] O. A. Shaikh, Z. Asghar, R. M. Aftab, S. Amin, G. Shaikh, and A. J. Nashwan, "Antimicrobial resistant strains of salmonella typhi: The role of illicit antibiotics sales, misuse, and self-medication practices in pakistan," *Journal of Infection and Public Health*, vol. 16, no. 10, pp. 1591–1597, 2023.

[3] K. K. Khudair and M. F. Ghasemi, "Phenotype and genotypic characteristics of isolated clinical salmonella typhi strains from children with diarrhea," *Medical Journal of Babylon*, vol. 21, no. Suppl 1, pp. S58–S63, 2024.

[4] P. B. Gathura, B. Obura, E. Muthuma, N. Mariach, B. Mkanga, R. Koigi, and S. Lolokote, "Situational analysis of safety of animal-source foods, fruits and vegetables in kenya," 2020.

[5] M. E. S. Guerra, G. Destro, B. Vieira, A. S. Lima, L. F. C. Ferraz, A. P. Hakansson, M. Darrieux, and T. R. Converso, "Klebsiella pneumoniae biofilms and their role in disease pathogenesis," *Frontiers in cellular and infection microbiology*, vol. 12, p. 877995, 2022.

[6] N. J. Sweeney, P. Klemm, B. A. McCormick, E. Moller-Nielsen, M. Utley, M. A. Schembri, D. C. Laux, and P. S. Cohen, "The escherichia coli k-12 gntp gene allows e. coli f-18 to occupy a distinct nutritional niche in the streptomycin-treated mouse large intestine," *Infection and immunity*, vol. 64, no. 9, pp. 3497–3503, 1996.

[7] S. B. Kurniawan, M. F. Imron, C. E. N. C. E. Chik, A. A. Owodunni, A. Ahmad, M. M. Alnawajha, N. F. M. Rahim, N. S. M. Said, S. R. S. Abdullah, N. A. Kasan, *et al.*, "What compound inside biocoagulants/bioflocculants is contributing the most to the coagulation and flocculation processes?," *Science of the Total Environment*, vol. 806, p. 150902, 2022.

[8] R. R. B. Vougat Ngom and H. S. Foyet, "In vitro antibacterial, non-cytotoxic and antioxidant activities of boscia senegalensis and tapinanthus dodoneifolius, plants used by pastoralists in cameroon," *Pastoralism*, vol. 12, no. 1, p. 13, 2022.

[9] "Evans, william charles. trease and evans pharmacognosy (16th edition) wb saunders company limited." https://books.google.com/books?hl=fr&lr=&id=l7pkTFyY428C&oi=fnd&pg=PT2&dq=Trease+and+Evans+Pharmacognosy+&ots=2pEvcs5dBj&sig=Iue8Ypc3LPocOcRU6NErwZ48EVUhttps://books.google.com/books?hl=fr&lr=&id=l7pkTFyY428C&oi=fnd&pg=PT2&dq=Trease+and+Evans+Pharmacognosy+&ots=2pEvcs5dBj&sig=Iue8Ypc3LPocOcRU6NErwZ48EVU.

[10] A. Nisca, R. Ștefănescu, C. Moldovan, A. Mocan, A. D. Mare, C. N. Ciurea, A. Man, D.-L. Muntean, and C. Tanase, "Optimization of microwave assisted extraction conditions to improve phenolic content and in vitro antioxidant and anti-microbial activity in quercus cerris bark extracts," *Plants*, vol. 11, no. 3, p. 240, 2022.

[11] S. M. Lai, D. Sudhahar, and K. Anandarajagopal, "Evaluation of antibacterial and antifungal activities of persicaria chinensis leaves," *Int. J. Pharm. Sci. Res*, vol. 3, pp. 2825–2830, 2012.

[12] M. I. I. Abdoulahi, M. D. Yanick Kevin, T. Y. Lauve Rachel, H. H. Habibou, B. Sahabi, A. S. Abdelkader, F. F. Boyom, and I. A. Tidjani, "Antibacterial activity of eight medicinal plants from the traditional pharmacopoeia of niger," *Journal of Tropical Medicine*, vol. 2023, no. 1, p. 6120255, 2023.

[13] A. Aliyu, A. Musa, M. Abdullahi, A. Oyewale, and U. Gwarzo, "Activity of plant extracts used in northern nigerian traditional medicine against methicillin-resistant staphylococcus aureus (mrsa)," *Nigerian Journal of Pharmaceutical Sciences*, vol. 7, no. 1, pp. 1–8, 2008.

[14] O. O. Temitope, "Bioactivity and phytochemical screening of nigerian medicinal plants growing in ondo and ekiti state against bacterial isolates from pediatrics hospital," *Journal of Advances in Medical and pharmaceutical Sciences*, vol. 4, no. 2, pp. 1–14, 2015.

[15] A. Maroyi, "Review of medicinal uses, phytochemistry and pharmacological properties of boscia senegalensis," *Journal of Pharmaceutical Sciences and Research*, vol. 11, no. 9, pp. 3355–3361, 2019.



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